in the NH_2- and -COOH terminal regions. These changes lead to frame shifts that are not compatible with a conserved ORF, therefore an assumption is that the sequence obtained from the 2.7 kb fragment represents a pseudogene (*FUT2P*). After subcloning of ETHs3 BamHI digests, the hybridizing sequences contained in the 8.2 kb EcoRI fragment were identified. The sequence of the subclones obtained represents a 1023 bp ORF and is 85% identical at the nucleotide- and 83%- identical at the amino acid level to the human *FUT2* sequence. Many differences in the NH_2- and -COOH terminal regions were observed between the porcine *FUT2* sequence and the *FUT2P* sequence derived from the 2.7 kb fragment. The predicted amino acid sequence corresponds to the partially determined amino acid sequence of the porcine *Secretor* enzyme (Thurin and Blaszczyk-Thurin, 1995). The porcine *FUT1, FUT2,* and *FUTP* sequences obtained were submitted to GenBank and have accession numbers U70883, U70881 and U70882, respectively. The *FUT1* and *FUT2* genes have highly homologous sequences. This has to be considered in, for example, primer development. Furthermore, *FUT1* and *FUT2* enzyme activity need to be differentiated in further studies.

Example 6: Identification of M307 and M857 Mutations and Characterization of M307

DNA was isolated from porcine nucleated cells according to standard procedures. Direct sequencing of porcine *FUT1* and *FUT2* sequences and their flanking regions in animals of different *ECF18R* genotypes (*Bb, bb*) resulted in the identification of two G→A transitions at positions 307 and 857 (termed *M307* and *M857*, respectively) of the *FUT1* ORF. The *M307* transition eliminates a restriction site for the enzyme CfoI. Amplification of DNA isolated from porcine nucleated cells was performed according to standard procedures with primers P6 and P11 (3 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 56°C and 30 sec at 72°C, followed by a 7 min final extension at 72°C) followed by CfoI digestion and separation on a 3% agarose gel resulted in a restriction fragment length polymorphism (RFLP). Homozygous $M307^{AA}$ animals showed 2 bands (93- and 328-bp fragments). Homozygous $M307^{GG}$ animals showed 87-, 93-, an 241-bp fragments. Heterozygous animals showed all four fragments.

Example 7: Characterization of Mutation M857

The M857 mutation is a transition that eliminates an AciI site. Primer PBEST was designed to mismatch two additional AciI sites at positions 866 and 872. PCR with primers P7 and PBEST (3 min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 56°C and